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# Development and characterization of micellar systems for application as insect repellents

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ABSTRACT

N,N-diethyl-meta-toluamide (DEET) is a widely used insect repellent due to its high efficacy. In this work, micellar systems based on poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer were developed and studied for the purpose of controlling the release and cutaneous permeation of DEET, using concentrated solutions of the copolymer Pluronic F127 to form thermoreversible gels. The formulations presented thermoreversible gelation above 5 °C and altered rheological behavior at 15 and 25 °C. The presence of the drug drastically changed the sol–gel transition temperatures. The micrographs suggest that DEET induced the formation of anisotropic structures, and Maltese Crosses were observed. The formulation containing 10 wt% DEET and 15 wt% Pluronic F127 presented sustained drug release for up to 7 h. DEET release profile followed the Higuchi kinetics model. There was a reduction of approximately 35% in the amount of DEET absorbed through the skin after 6 h. About 62% of DEET from the formulation consisting of Pluronic F127 and DEET remain retained on the skin. The anisotropic structure may constitute a barrier to diffusion and thereby controlling the drug release effectively. These tests suggest that the tested samples exhibit safety profile greater than some commercially available products.



## 1. Introduction

In the world, millions of people suffer each year from diseases transmitted by mosquitoes, which are considered to be the main cause of contagious diseases (Tolle, 2009). Besides these diseases, insect bites and stings cause local discomfort and irritation. Although more common in tropical and subtropical regions, outbreaks of insect-borne disease can occur any place in the world. Public strategies which aim vector control and reduce disease incidence can lower the risk of epidemics. However, individual protection is still one of the main forms of prevention and is particularly important because of the absence of vaccines and curative treatments for the great majority of these diseases (Bissinger and Roe, 2010; Impoinvil et al., 2007).

Among the commercial repellents, products containing N,N-diethyl-meta-toluamide (DEET) (Fig. 1) have been used for decades due to its good repellent activity (Sudakin and Osimitz, 2010). However, there are reports, although rare, of severe adverse reactions to products containing DEET (Antwi et al., 2008; Masson, 2011). These events are usually related to the presence of the molecule in the bloodstream, since DEET is capable of passing through the cutaneous barrier, reaching deeper skin layers by diffusion and entering the blood vessels quickly (Winter, 2005).

Polymeric micelles can be used in drug carrier systems as well as for guided release due to their high encapsulation capacity. Pluronic F127 or Poloxamer 407 is a commercial block copolymer with A–B–A architecture, a symmetrical structure composed of a central segment of poly(propylene oxide) (PPO) and two peripheral segments of poly(ethylene oxide) (PEO). Therefore, this block copolymer presents surfactant activity and it is widely used in formulating pharmaceutical products because of its thermoreversible gelation characteristic, allowing the drug to remain at the administration site for longer periods and enhancing its therapeutic efficacy (Alexandridis et al., 1996; Pepic et al., 2004; Ruel-Gariepy and Leroux, 2004; Sharma and Bhatia, 2004; Sharma et al., 2008). The gelation phenomenon of concentrated solutions of Pluronic F1227 have been attributed to the formation of ordered structures such as liquid crystals with lamellar, cubic or hexagonal arrangements (Ivanova et al., 2000; Liu and Chu, 2000). Recently, gels based on Pluronic F127 have been receiving special attention for the

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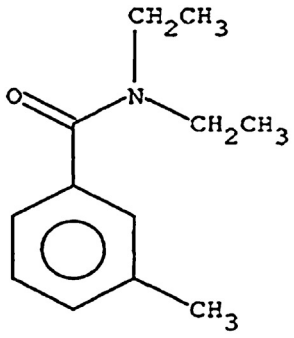

| | |
|---|---|
| $\log k_{oct}$ | 2,02 |
| MW | 191,27 Da |
| ρ(30°C) | 0,984g/cm³ |
| $P_{vp}$ (30°C) | 0,00267 torr |
| $bp_{760}$ | 288-292°C |

**Fig. 1.** Structure and physical properties of DEET (Santhanam et al., 2005).

development of dermal and transdermal release systems, to hasten or retard the permeation of drugs through the skin (Antunes et al., 2011). The thermoreversible characteristic and release pattern make Pluronic F127 a good candidate to be a carrier of drugs through various administration routes (Bivas-Benita et al., 2004; Kojarunchitt et al., 2011).

The attempt to reduce the permeation rate of synthetic repellents through the skin is a challenge that must be overcome during the development of new repellent formulations, so as to minimize the risk of cutaneous absorption of molecules such as DEET. In this context, the application of polymers to modify the drug diffusion trough the formulation's vehicle, or for microencapsulation of active substances to control their release, is of fundamental importance for the development of new formulations.

## 2. Experimental

### 2.1. Materials

The synthetic insect repellent DEET (N,N-diethyl-meta-toluamide) (Clariant S/A, São Paulo, Brazil) cellulose acetate membrane (pore size of 0.2 μm and thickness of 43 mm) (Sigma, MO, USA); and the poly(ethylene oxide)–poly(propylene oxide) block copolymer, Pluronic F127 (Sigma, MO, USA) were used in this study.

### 2.2. Methods

#### 2.2.1. Preparation of the formulations

The formulations were prepared by adding a mass of the solid triblock copolymer to an appropriate amount of ultrapure water. The polymer/water mixture was then cooled to 2 °C until all the solids were dissolved for 24 h. Appropriated amounts of DEET were added to the systems and then the samples were processed in an Ultra-Turrax® (IKA, model T10) homogenizer at 8000 rpm for 2 min. All the samples were kept in a refrigerated bath at 4 ± 2 °C. The concentrations of polymer and repellent are expressed as percentage by weight (wt%) (Table 1).

#### 2.2.2. Rheological characterization of the micellar systems

The dynamic and steady-state analyses were carried out in a rotational rheometer Rheostress 600 (Haake, Karlnuhe, Germany). Measurements were performed in a stainless steel cone-plate geometry, with a cone diameter of 35 cm and angle of 1° and a gap of 0.052 mm. The temperature was controlled by a Phoenix II cooling and heating system (precision of 0.1 °C). In order to minimize water evaporation, a solvent trap has been used during rheological experiments. All samples were analyzed at three temperatures (5, 15 and 25 °C). The reliability of the measurements was assured by obtaining the rheograms in triplicate at all temperatures studied.

**Table 1**
Formulations prepared and the ratio between $G'/G''$ at three temperatures studied.

| Formulation | Composition | | Temperature | $G'/G''$ |
|---|---|---|---|---|
| I | Pluronic F127 | 12% | 5 °C | 5.9 |
| | DEET | 5% | 15 °C | 6.4 |
| | $H_2O$ qsp 20 mL | | 25 °C | 3.6 |
| II | Pluronic F127 | 12% | 5 °C | 4.3 |
| | DEET | 7% | 15 °C | 4.3 |
| | $H_2O$ qsp 20 mL | | 25 °C | 4.2 |
| III | Pluronic F127 | 12% | 5 °C | 1.7 |
| | DEET | 10% | 15 °C | 4.3 |
| | $H_2O$ qsp 20 mL | | 25 °C | 4.2 |
| IV | Pluronic F127 | 15% | 5 °C | 5.4 |
| | DEET | 10% | 15 °C | 4.5 |
| | $H_2O$ qsp 20 mL | | 25 °C | 5.6 |

The rheological characterization was performed in four steps:

(a) The flow experiments were carried out in a shear rate range between 0.01 and 100 $s^{-1}$. The flow curves were determined with two consecutive continuous shear rate ramps of 0.01–100 $s^{-1}$ with ascending and descending cycle.
(b) The sol-gel transition was determined by observing the viscosity variation under heating at a rate of 3 °C $min^{-1}$, in the temperature interval of 5–40 °C. The experiments were conducted at a constant shear rate of 100 $s^{-1}$.
(c) Oscillatory tests were conducted to determine the region of linear viscoelasticity with variation of deformation from 0.1 to 600% at a frequency of 1.0 Hz.
(d) The viscous modulus ($G''$) and elastic modulus ($G'$) were determined as a function of the variation of frequency from 0.1 to 90 Hz, under constant deformation of 0.3%.

#### 2.2.3. Polarized light microscopy

An Olympus BX50F-53 microscope was used in this study. A droplet of each sample was pressed between the slide glass and the coverslip so as to have thickness of only a few microns. Then, the slide system was placed in a sample holder and analyzed under polarized light, at room temperature (25 ± 2 °C).

The micrographs were captured by a Nikon Coolpix 5400 camera coupled to the ocular lens and were digitized using a video capture card, which transmitted the data directly to a personal computer.

#### 2.2.4. Quantification of DEET by high performance liquid chromatography (HPLC)

We initially constructed a calibration curve using a Jasco MDS-2010 Plus chromatograph, with an NST C18 column (25 cm) and a PDA detector, using the method developed and validated by Kasichayanula et al. (2005). The mobile phase was composed of methanol and water (70:30), with flow of 1 mL $min^{-1}$, loop of 20 μL,

PDA detector at 240 nm, and retention time of 5.01 min. The calibration curve was obtained by the ratio of the area under the curve in function of the concentration of the injected solution. Five solutions were tested with theoretical concentrations of 15.0, 25.0, 35.0, 45.0 and 50.0 μg mL$^{-1}$. The analyses were conducted in triplicate.

*2.2.5. Solubility, sink conditions and in vitro release of DEET*

A vertical diffusion system was assembled for the *in vitro* release studies. The system was composed of a donor compartment and receptor compartment between which a semi-synthetic membrane composed by cellulose acetate was placed. The diffusion area was 1.54 cm$^2$ and the volume of the receptor compartment was 50 mL. In order to ensure the sink conditions, the receptor compartment was filled with phosphate buffered saline (PBS) buffer pH 7.4 and 30% ethanol and maintained at 32 °C under constant stirring. Then, 0.5 g of sample was applied in the donor compartment. The PBS composition was 8.0 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4$ and 0.24 g $KH_2PO_4$.

At predefined intervals (30 min, 1, 2, 3, 4, 5, 6 and 7 h), aliquots of 500 μL were removed for analysis and then replaced with fresh receptor solution. The samples were analyzed by HPLC. The transport of the DEET through the membrane was defined as the quantity of DEET released per unit of time.

The zero-order, Higuchi (pseudo zero order) and first-order kinetic models were applied in the release profiles presented at the end to obtain the most appropriate model for each profile. The choice of the best model was made based on the linear correlation coefficient ($r$) obtained in each linear regression analysis.

Zero-order kinetics is represented by Eq. (1),

$$Q_t = Q_0 + K_0 t \quad (1)$$

where $Q_t$ is the quantity of the drug released at time $t$, $Q_0$ is the initial quantity of the drug in the solution and $K_0$ is the zero-order release constant and $t$ is the time. First-order kinetics is described by Eq. (2),

$$\ln Q_t = \ln Q_0 + K_t t \quad (2)$$

where $Q_t$ is the amount of the drug released at time $t$, $Q_0$ is the initial quantity of the drug in the solution and $K_t$ is the first-order release constant (Costa and Sousa Lobo, 2001). The Higuchi model is described by Eq. (3),

$$Q_t = K_h + \sqrt{t} \quad (3)$$

where $Q_t$ is the quantity of the drug released at time $t$ and $K_h$ represents the Higuchi release constant (Higuchi, 1961).

*2.2.6. In vitro skin permeation study*

We performed the skin permeation assay into two stages: The first one consisted of the skin permeation test, which evaluated the amount of DEET that crossed the skin barrier, reflecting a possible absorption of DEET into the bloodstream. The second stage consisted of the retention test in the *stratum corneum*/*epidermis*/*dermis*, assessing whether DEET was retained in the skin and thus, might exert a residual topical effect.

Skin permeation studies were carried out using Franz diffusion cells. The cell body was filled with 8 mL of a receptor phase consisting of PBS pH 7.4, according to the method published by Karr et al. (2012) and Kasting et al. (2008). The receptor medium was constantly stirred and thermostated at 32 °C throughout the experiments. Approximately 200 mg of formulations I, IV and the control samples were placed in the donor chamber onto the *stratum corneum* of abdominal mouse skin, in occlusive conditions. The diffusion area was 1.77 cm$^2$.

Samples of 200 μL were taken 30 min, 1, 2, 4 and 6 h after application of formulation and replaced with fresh receptor solution. All samples were submitted to HPLC analysis.

After 6 h of the experiment, the Franz cells were disassembled, and the excess formulation was removed from the surface of skins. The treated areas of skin segments were triturated and disposed in different tubes. 5 mL of methanol were added to the tubes and the extraction of DEET was proceeded for 24 h. The samples were filtered by disposable filter unit (0.45 mm) and analyzed by HPLC.

*2.2.7. Data analysis*

All the statistical tests were repeated three times and expressed as the mean ± SE using Origin Pro 8 (OriginLab, USA) software.

## 3. Results and discussion

### 3.1. Rheological characterization of the micellar systems

*3.1.1. Flow experiments*

Flow experiments were conducted with the micellar systems containing DEET and Pluronic F127 and the resulting rheograms were compared with those of the control sample.

Each formulation presented lower viscosity values at 5 °C in comparison to 15 and 25 °C, at which they showed aspects similar to a gel. At 5 °C, all samples presented a Newtonian behavior. At 15 and 25 °C, all the systems presented a shear thinning behavior and their viscosities depended on the temperature and shear rate (Fig. 2). It can be explained by the fact that the PPO block is only soluble in water up to 15 °C. Above this temperature, the hydrogen bonds between the PPO block and water molecules become unstable, leading to desolvation of the block, favoring intermolecular interactions of the polymer chains and micellization (Shvartzman-Cohen et al., 2009). Such phenomenon explains the increase in viscosity with heating and the shear thinning behavior observed with the increase in the shear rate.

Above 15 °C, a decrease in viscosity with increased shear rate was observed for all the systems studied, indicating they are non-Newtonian systems. With the increase of shear rate, all formulations presented a Newtonian rheological behavior again, a phenomenon resulting from the orientation of the polymer chains in the direction of the shear tension applied, with deformation of the lyotropic domains and elongation of the micelles, which line up parallel to the shear force direction (Lopez-Barron et al., 2012).

*3.1.2. Determination of the sol–gel transition point*

The sol–gel transition was determined by the relation between viscosity and temperature, and the results shown in Fig. 3a and b

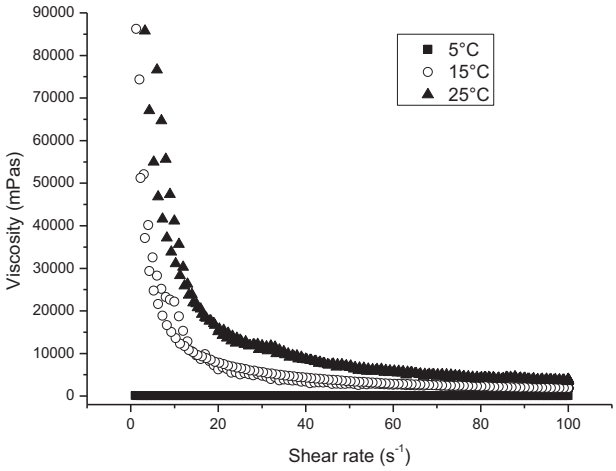


**Fig. 2.** Flow curves for formulation IV at 5 °C, 15 °C, 25 °C. All other formulations tested in this work presented a similar profile. Newtonian behavior at 5 °C. Shear-thinning behavior at 15 and 25 °C.

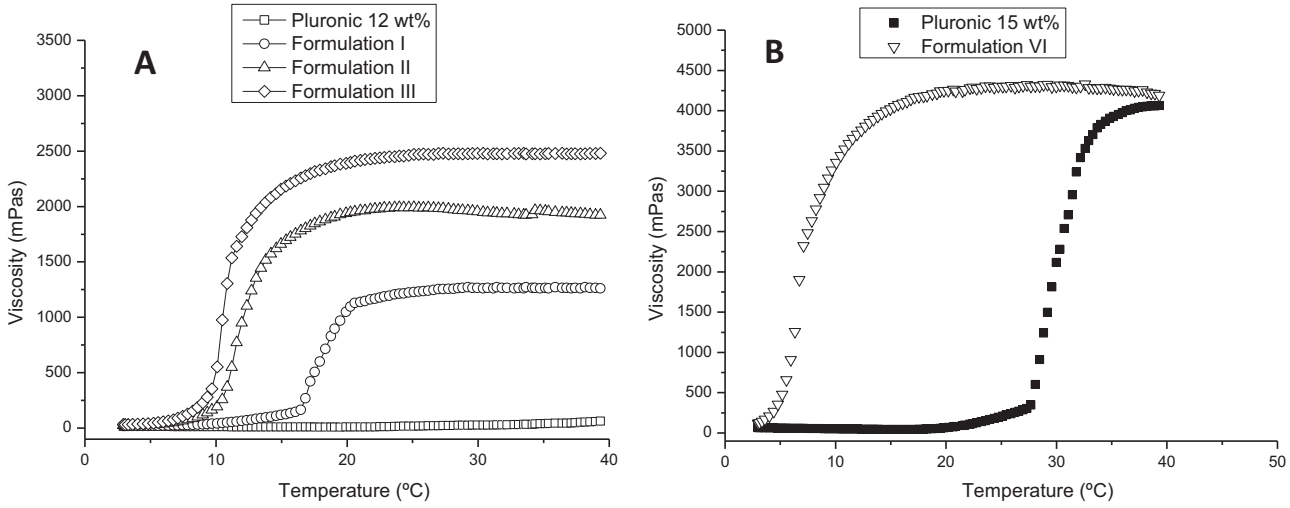


**Fig. 3.** Determination of the sol–gel transition for formulations I, II and III (A) and IV (B) and the control samples. Formulation I ($T_{gel}$ = 18 °C); formulation II ($T_{gel}$ = 12 °C); formulation III ($T_{gel}$ = 10 °C); formulation IV ($T_{gel}$ = 6 °C); 15 wt% Pluronic ($T_{gel}$ = 30 °C).

present the same profile as observed previously in the literature (Fernandez et al., 2009; Ma et al., 2008; Wei et al., 2002), showing curves with sudden viscosity increases, indicating a concentration-dependent gelation pattern.

At low temperatures, the systems had low viscosity, typical behavior of fluids. At these temperatures, unimers and a few micelles formed by polymer and DEET molecules coexist (Zhao et al., 2007). However, there was a sudden increase in viscosity within a narrow temperature range, in the region of the sol–gel transition, as also reported by other authors (Jiang et al., 2008; Lenaerts et al., 1987). In this region, the majority of micelles are formed and then, their solubility decreases with the dehydration of the PEO block, forming supramolecular aggregates (Zhao et al., 2007). At the end of the transition, a plateau was reached where the viscosity became independent of temperature, suggesting that after gelation there was no longer any variation of the viscosity values, indicating the absence of modification of the system's structure with increasing temperature, as concluded previously in the literature (Jiang et al., 2008; Tung, 1994).

The sol–gel transition temperature declined and the viscosity increased with increasing repellent concentration (from 7 to 10 wt%) in the systems. The presence of DEET probably induced micellar ordering at lower temperatures, favoring aggregate formation. There was also dependence of the viscosity observed at the plateau as a function of the DEET concentration, which was responsible for shifting the gelation temperature. The increase of DEET concentration led to a raise of the number of micellar aggregates and regions with crystalline organization in the formulations, increasing the probability of entanglement of the polymer chains and generating more viscous samples, thus shifting the curves to the left.

### 3.1.3. Strain sweep tests

To measure the viscoelastic properties of systems formulated with DEET and Pluronic F127, it is necessary to know the linear viscoelasticity region by determining the elastic modulus ($G'$) and viscous modulus ($G''$). For all the samples, under strain of 0.3%, $G'$ and $G''$ were independent of the deformation, indicating that the systems had reached the linear viscoelastic regime (Fig. 4). Within the linear viscoelastic regime, $G'$ was higher than $G''$, indicating the formation of a material with elastic characteristics superior to the viscous ones, typical behavior of gels (Basak et al., 2011; Koffi et al., 2006; Lopez-Barron et al., 2012).

Starting at around 10% of deformation, the material's structure started to rupture, causing reduction of the magnitude of the elastic and viscous components, indicating the end of the system's linear viscoelastic regime and the rupture of the material's structure. This behavior was repeatedly observed for all the other samples studied at the three temperatures applied. According to Youssry et al. (2008), this phenomenon is due to the formation of an intermediate structure that resists deformation until a critical point, above which the structure is broken, leading to alignment of the chains in the direction of the tension applied, at which point $G''$ becomes higher than $G'$.

### 3.1.4. Frequency sweep tests

This type of investigation enables classification of a dispersion as a diluted solution, concentrated solution (system of intertwined networks), weak gel or strong gel, according to the ratio between $G'$ and $G''$ (Speroni et al., 2009; Zhang et al., 2007).

These tests were performed under low strain (0.3%) to assure minimum disruption of the internal structures. In general, for all the samples evaluated $G'$ was higher than $G''$, indicating predominance of elastic characteristics over viscous properties for these systems, a classic behavior of gels (Zhang et al., 2007). Besides this,

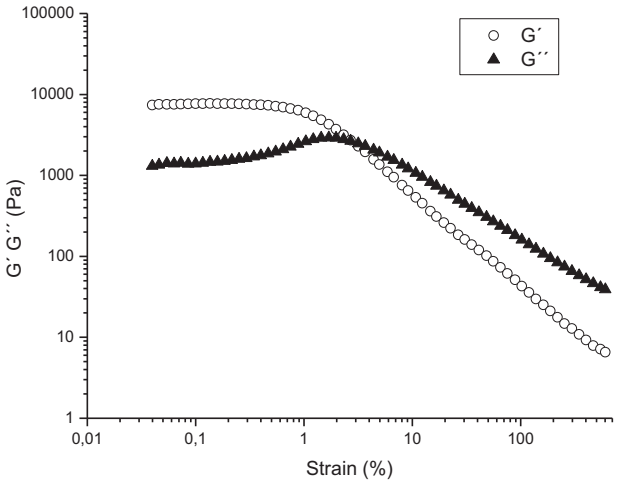


**Fig. 4.** Strain dependence of $G'$ and $G''$ determined by dynamic strain sweep measurement at 1.0 Hz for formulation IV at 25 °C. Linear viscoelasticity occurs at strains below 10%. All other formulations tested in this work presented a similar profile.

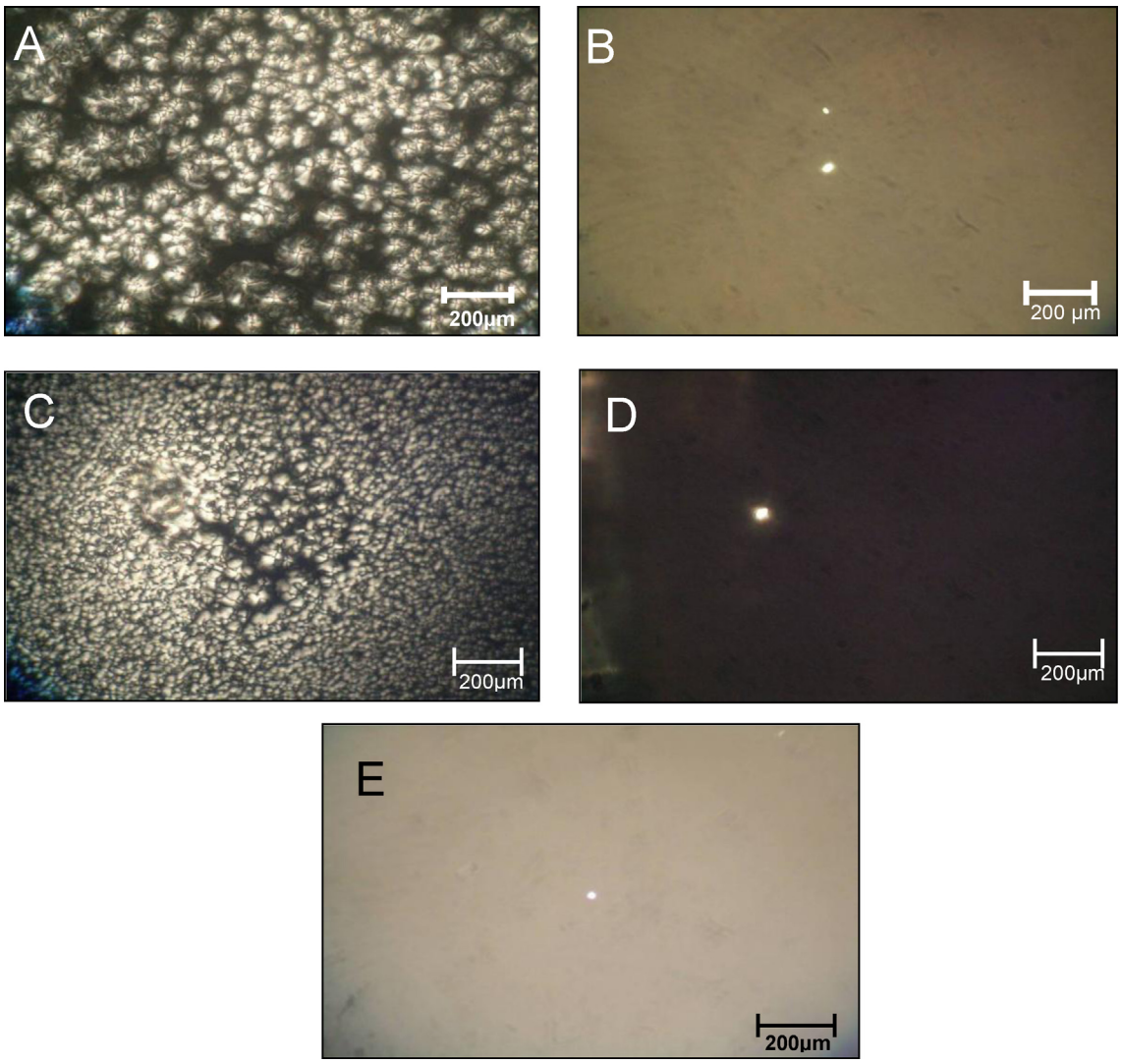


**Fig. 5.** Polarized light micrographs of formulation I (A) 12 wt% Pluronic F127 solution, (B) formulation IV, (C) 15 wt% Pluronic F127 which does not form gel at room temperature. (D) and gel constituted by 20 wt% Pluronic F127 (E). (Magnification 20×).

the increased temperature led to a rise in the magnitude of $G'$ and $G''$. This behavior was much more pronounced for $G'$, a result that can be associated with a greater interaction between the polymer chains at higher temperatures, strengthening the elastic network (Sturcova et al., 2010) (data not shown).

The values of $G'$ and $G''$ both increased with higher DEET and Pluronic F127 concentrations, indicating an increase in the mechanical resistance of the micellar systems. However, we observed a small and discrete dependence of the values of $G'$ and $G''$ with the frequency, but with continuing predominance of elastic behavior. Besides this, the $G'/G''$ ratio was less than 10 for all tested samples (Table 1), permitting classifying the systems studied as weak gels (Tokita and Nishinari, 2009). According to Guo et al. (2009) and Jimenez-Avalos et al. (2005), weak gels are dependent on the frequency and present a curve for $G'$ higher than that for $G''$, and do not present any crossing of $G'$ and $G''$. In elastic or true gels, the storage modulus does not vary with changing frequency.

### 3.2. Polarized light microscopy

To observe the anisotropic regions, we analyzed two formulations containing the Pluronic F127 and DEET (formulations I and IV) by polarized light microscopy and compared the results with micrographs of the corresponding systems without the drug. These analyses were carried out 24 h after preparing the samples. The results are shown in Fig. 5. Since the control samples, composed of 12 wt% and 15 wt% Pluronic F127 without DEET, did not form gels at room temperature, we studied gels formed by 20 wt% Pluronic F127 (Fig. 5e).

It can be seen that the presence of DEET induced the formation of organized anisotropic structures evidenced by "Maltese Cross" (Makai et al., 2003). On the other hand, the gel composed only by Pluronic F127 was isotropic, since its gelation process is based on the formation of an ordered packing of micelles into a cubic arrangement (Ivanova et al., 2000; Liu and Chu, 2000). That fact suggests that the gelation occurs by different mechanisms in these samples. The presence of DEET transformed the systems structures, probably by acting as a nucleating agent, inducing the formation of different anisotropic structures.

### 3.3. In vitro release study

Fig. 6 shows the drug release results for the formulations studied. It can be observed that the release of DEET from the formulation I was greater and faster than for the control. This fact can be explained by the drug's solubility: DEET is a hydrophobic

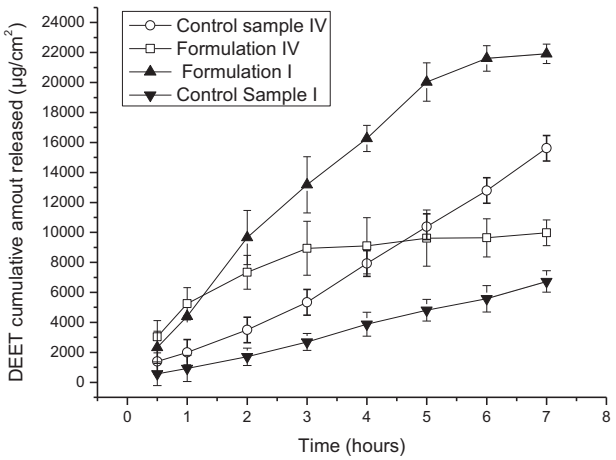


**Fig. 6.** Cumulative *in vitro* release of DEET from formulations I and IV and their respective control samples ($n = 3$).

compound, highly soluble in ethanol. In this experiment, this solvent was a constituent both of the receptor solution and the control solution. However, in the receptor solution it was only present at 30%, *versus* 100% in the control solution. Therefore, the drug does not tend to leave the formulation in which it is freely soluble to the receptor solution in which it is less soluble, because it is more thermodynamically stable. For this reason, the composition of the receptor media and the control sample can influence the release profiles observed.

The presence of Pluronic 127 can cause osmotic imbalance in the system, so that water molecules will travel upward through the membrane. This would lead to dilution of the system and separation of the micelles, thus destabilizing the formulation. This phenomenon would favor the diffusion of the drug through the membrane. Such dilution caused by osmotic imbalance could also alter the polymer's concentration to levels below the critical micelle concentration, resulting in a loss of gel structure and leading to disassociation of the micelles into free polymer chains (Chun et al., 2005; Kojarunchitt et al., 2011). Therefore, the drug, which was enclosed in the micelles, would suddenly become free in the medium, generating a burst effect upon release (Qu et al., 2009; Ye et al., 2008).

Besides this, it can be seen that the release of the DEET was slower, lasting up to 7 h. This observation indicates the occurrence of rapid initial release followed by a slower and more prolonged release pattern after five hours. Since the micellar system contains liquid crystalline regions composed of ordered micelles with crystalline and amorphous regions, the drug can be located in either of these regions. DEET molecules encapsulated in the micelles are in dynamic balance with those located in the aqueous phase. Therefore, the DEET molecules preferentially located in the amorphous regions and outside the micelles are first released, explaining the greater release profile observed for this formulation. The DEET molecules located inside the crystal regions are released more slowly. Thus, the crystalline structure observed for this sample can pose a barrier to diffusion, exercising a reservoir effect by effectively controlling the drug release (Fig. 5). It was also observed by Makai et al. (2003), which observed that the lamellar structures were better able to control the *in vitro* drug release.

We compared the DEET release patterns of the two formulations to understand the influence of increasing quantities of Pluronic F127 and DEET in the systems (Fig. 6). Note that the formulation IV was more effective in controlling and sustaining the drug release during the period of the experiment. These results can be explained by the structural differences of the systems in terms of crystalline regions, which hinder the diffusion of drugs. The micrographs suggest that the formulation I (Fig. 5a) presents a less dense structure than formulation IV (Fig. 5c). The apparent higher density anisotropic regions might hamper diffusion of the drug through the formulation retarding the rate of drug release. However, this conclusion should be confirmed by complementary structural investigations, since the density of Maltese Crosses depends on the sample preparation and on the thickness of the sample between the slide glass and the coverslip.

Both formulations presented the highest linear correlation coefficients ($r$) when the Higuchi model was applied (Table 2). Higuchi kinetic model follows the principles of Fick's law of diffusion, *i.e.*, there will be release the drug until equilibrium is reached between the drug concentrations inside and outside the formulation (Higuchi, 1963; Phan et al., 2011; Ye et al., 2011). Although the release speed became slower with increased concentration of Pluronic F127 and DEET, the release kinetics remained the same.

### 3.4. *In vitro skin permeation studies*

When assessing the possible skin permeation of DEET, we noted that for all samples, detectable amounts of DEET were able to cross all layers of the skin and reach the receptor solution after a few hours (Fig. 7a). The increase in the concentration of Pluronic F127 and DEET clearly decreases the amount of drug permeated in comparison with the control sample. This phenomenon may be attributed to the presence of anisotropic regions in the structure of formulations, which may control the drug permeation.

In addition, data from the rheological studies corroborate for the conclusion that the more viscous systems are more efficient in controlling the permeation of DEET. The viscosity values and $G'$ and $G''$ for formulation IV were greater than to those exhibited by formulation I. This fact indicates the formation of a stronger elastic network that might act as a barrier to diffusion of the drug through the skin.

The control sample composed of an ethanolic solution of DEET presented higher drug permeation profile in comparison with the two formulations tested. It is known that the great majority of repellent products commercially available are ethanolic solutions (Karr et al., 2012; Tuetun et al., 2005). Many authors state that ethanol is able to stimulate the cutaneous absorption of many lipophilic drugs primarily inducing the solubilization of the lipid constituents of the skin and dehydrate the skin tissue forming microfissures and pores in the *stratum corneum* (Karr et al., 2012; Van der Merwe and Riviere, 2005; Watkinson et al., 2009). DEET

**Table 2**
Formulation and release kinetic parameters.

| Formulation | Zero order model | Higuchi model | First order model |
|---|---|---|---|
| I | $R^2 = 0.9553$<br>$r = 0.9773$<br>$y = 3175.3x + 2362.4$ | $R^2 = 0.9892$<br>$r = 0.9945$<br>$y = 10985x - 5803$ | $R^2 = 0.8177$<br>$r = 0.9043$<br>$y = 0.3162x + 8.1718$ |
| VI | $R^2 = 0.7784$<br>$r = 0.8822$<br>$y = 937.88x + 4521.5$ | $R^2 = 0.893$<br>$r = 0.9449$<br>$y = 3415.3x + 1807.3$ | $R^2 = 0.6772$<br>$r = 0.8229$<br>$y = 0.1458x + 8.387$ |

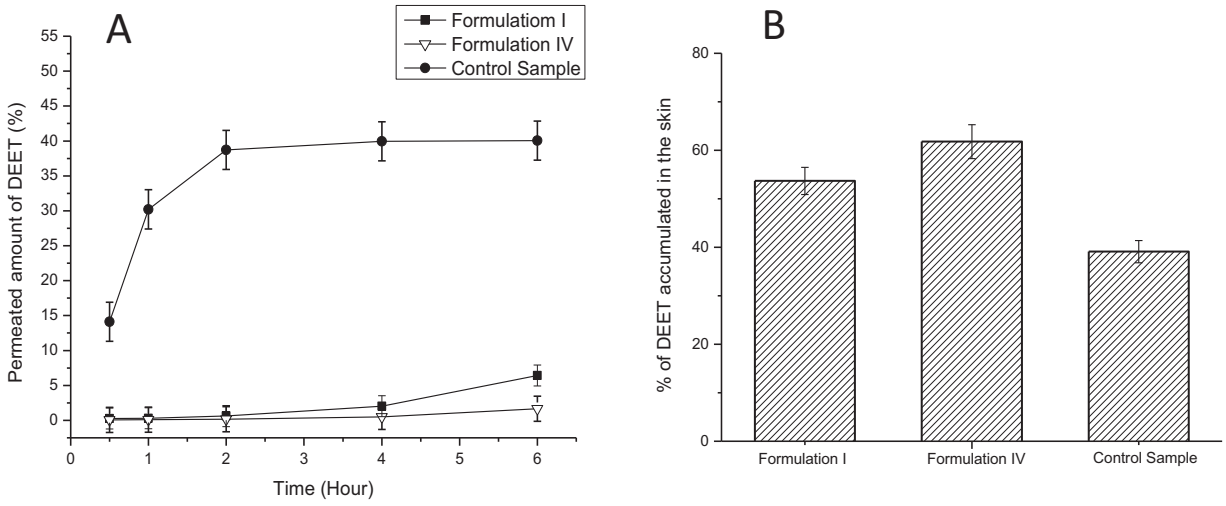


**Fig. 7.** (A) *In vitro* DEET permeation profiles through mice skin after topical application of the formulations studied and control sample. The cumulative amount released was plotted against time ($n = 3$). 6.43% of DEET from formulation I permeated the skin, while formulation IV showed no more than 1.66% of the amount of DEET permeated. In comparison, the control sample showed 39.4% of DEET permeation. (B) Percent of the total skin retention of DEET after 6 h of topical application of formulations. The study was performed *in vitro* using Franz diffusion cells on mice skin ($n = 3$).

is a small molecule and with moderate lipophilicity (Fig. 1), thus penetration through the *stratum corneum* would occur rapidly. In this work, there was a reduction of 33–38% of the amount of DEET absorbed through the skin (Fig. 7a). Thus, these tests indicate that both formulations tested had a greater safety profile than repellent products commonly found on the market which are ethanol-based formulations.

At the end of permeation experiments, the presence of DEET in the skin of mice was determined (Fig. 7b). In general, the tested formulations provided greater amount of DEET retained on the skin compared to control. About 62% of DEET contained in the formulation IV remained on the skin without breaching it, when comparing to 40% obtained for the control sample. This is a beneficial effect since it may provide a residual protection provided from this product even after its removal from the skin surface, which could benefit practitioners of outdoor activities, people who might perform military incursions into forests and other individuals constantly exposed.

## 4. Conclusions

Stable micellar systems containing 5–10 wt% DEET and 12 and 15 wt% Pluronic F127 were obtained. All the formulations presented thermoreversible gelation, with declining viscosity as the shear rate increased, characterizing shear thinning behavior regarding flow, typical of semi-solid polymer systems.

The presence of the drug drastically altered the rheological behavior of the systems based on Pluronic F127 and DEET. The sol–gel transition temperature was drastically shifted to lower values in a phenomenon depending on the concentration of DEET and Pluronic F127. The rheological tests permitted classifying the systems as weak gels.

The light polarized micrographs suggested that the presence of DEET was able to induce the formation of anisotropic structures, with structures such as Maltese Cross, suggesting the drug might act as a nucleating agent.

Formulation IV presented slower and more sustained release for a period of 7 h, which might suggest that formulations with higher density of anistropic regions would be better able to control the amount of drug released. The DEET release profile of both formulations followed the Higuchi kinetic model, according to which this release is governed by Fick's diffusion law.

All tested formulations exhibited a considerable reduction in skin permeation and increased retention of DEET into the skin when compared with the control sample. This fact suggests an improvement not only in the effectiveness of micellar systems based on Pluronic F127 as DEET but also in their safety profile, making them promising products for developing optimized topical repellent formulations.

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